Leaf senescence: Physiology and molecular biology

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Leaf senescence although deteriorative in nature, has been recognized as the last phase of the organ's development, a highly ordered process regulated by genes known as senescence associated genes (SAGs). Till now, more than 30 SAGs have been isolated, cloned and characterized. The leaf when young and mature, accumulates nutrients and exports them to growing parts of the plant during senescence. The macromolecular degradation and their transport during senescence are reported to be strictly controlled by genes. The genes are also reported to actively participate in energy metabolism and supply metabolic energy for the transport of nutrients. Through genetic regulation, the senescing leaves maintain cellular integrity and potential not only for nutrient transport, but also for effective transcription and translation of proteins. Although the genes specific for induction of senescence have not yet been precisely identified, the down-regulation of photosynthetic genes has been proposed to be the possible signal for up-regulation of SAGs and induction of senescence. Leaf senescence is recognized as a process following a programmed cell death (PCD). The regulatory elements of some of the SAGs are characterized and their response to senescence inducing factors indicates scope for further studies on the molecular mechanism of signal response coupling during foliar senescence.

A special section consisting of several articles on biology of aging appeared in the 25 May 1998 issue of *Current Science*. However, all these articles mostly describe data relating to biochemistry and molecular biology of aging and senescence in animal systems only. Aging and senescence are used interchangeably in case of animals but in plants, the process of senescence is better defined as an active process and is well differentiated from aging which is considered to be passive time-dependent degeneration. The basic molecular mechanism of senescence both in plant and animal systems may be the same. The process involves expression of specific genes.

Since senescence constitutes an internally regulated developmental process, it has a logic in plant life and therefore, carries significant physiological implications. A programmed senescence, is basically an adaptive mechanism and the death, its consequence, therefore, takes place on the organism's own terms. In nature, senescence in leaves is the best example that fits into this concept. Leaf senescence has extensively been investigated in the last few years. The process, however, is not only concerned with death but involves several events associated with massive mobilization of nutrients in a highly ordered and regulated manner from senescing leaves to new leaves, developing fruits, seeds and buds, thus contributing to the nutrient cycling. The senescing leaves carry out these events and therefore remain viable and active. Although there are limits for generalization and extrapolation of the mechanism of leaf senescence in understanding the process in whole plants, the study however, provides vital clues to the knowledge of basics of senescence as a process.

Senescence of a leaf is temporally regulated in a coordinated manner[1,2]. The cellular components in the senescing leaves experience a sequential dismantling with a perfect order[3,4]. Although the process operates under the active control of genes, it is known to be modulated by environmental signals[5]. The precise triggering mechanism of leaf senescence still remains unclear but it is proposed to be induced by intrinsic and environmental factors[5,6]. In green leaves, the process is mostly characterized by a loss in total chlorophyll[7]. In addition, the degradation of macromolecules, namely proteins, nucleic acids, lipids and a decline in photosynthesis, remobilization of nutrients and the dismantling of cellular organelles are other major events associated with the process[3,6]. The genes regulating these events are known as senescence associated genes (SAGs)[5,8]. More than 30 SAGs are isolated, cloned and characterized in different plant systems.

This review describes the expression of the SAGs associated with macromolecular degradation and mobilization of nutrients from senescing leaves. It also describes the genes for maintaining the viability of senescing cells. The review very briefly covers findings on chloroplast degradation during leaf senescence and down-regulation of the photosynthetic genes as the possible factor for induction of leaf senescence. Regulation of senescence-associated genes, with particular reference to study of their promoters are also discussed. Some of the questions in this area, still unanswered, are addressed in the concluding section.

Genes for macromolecular degradation during leaf senescence

The turnover of macromolecules particularly proteins is a common occurrence during plant growth and development. Although degradation of macromolecules is one of the major events that occurs during leaf senescence, the process nevertheless involves the synthesis of RNA and proteins *de novo*[1,6]. Macromolecular degradation of senescing leaves is a pre-requisite for making transportable nutrients that are subsequently remobilized to young and expanding organs of the plants. Senescence-induced protein breakdown that has been well reported in many plant systems, results in availability of transportable nitrogen[6]. The precise mechanism and the type of specific proteases involved in protein breakdown, however, are yet to be known but the possible participation of cysteine proteases and aspartic proteases in the process has been suggested[9–11].

The degradative enzymes may not be very specific to any developmental sequence including senescence. For example, most of the enzymes that participate in the degradation of macromolecules for reserve mobilization during germination also participate in degradation during senescence[11–14]. During tomato leaf senescence, Drake *et al.*[15] have isolated two cDNA clones that exhibit homology with the genes for the cysteine proteinases. These genes for synthesis of the proteases are also known to express during seed germination. Similarly, the cDNA clones isolated from *Arabidopsis* during senescence exhibit sequence identity with cysteine protease having a high degree of homology with oryzain y and aleurain[8], the proteases well known for their action in the degradation of reserve proteins for the mobilization of nitrogen during seed germination[16,17].

Smart *et al.*[9] have identified and characterized several genes associated with leaf senescence of maize plants. Senescence shows enhanced expression of two genes, one exhibiting sequence homology with cysteine proteases oryzain y from rice and the other with the protein-processing enzyme of castor bean seeds. It is likely that these genes participate in degradation of proteins and then mobilization of the breakdown products in transportable form from senescing leaves to other parts of the plants. These observations thus suggest that the genes for proteases for macromolecular degradation may not be specific to senescence. In fact, a level of expression of the genes is also observed in young and mature leaves, however, with their enhanced expression during senescence[5]. In addition to the expression of genes for the proteases, senescence-induced enhanced expression of genes for synthesis of nucleases[18] and lipases[19] for degradation of nucleic acids and lipids, respectively, are also reported. The SAGs relating to macromolecular degradation are not supposed to be associated with senescence induction. The genes express only after initiation of the process[9].

Participation of genes for export of nutrients

A leaf during its rapid development acts as a strong sink drawing nutrients from other sources of the plant and is converted to a source of nutrients when it becomes mature with full photosynthetic establishment. Maturity of the leaf is likely to send signal(s) for a decline in photosynthesis, induction of senescence and subsequent mobilization of resources to other growing regions. A functional shift from photosynthesis to resource mobilization in senescing leaves significantly contributes to the optimization of resource utilization by the whole plant. The leaf, at late stage of senescence with rapid loss in photosynthetic activity and relatively high respiratory rate obviously becomes obsolete and therefore, becomes a respiratory burden for plants. The plant, therefore, rejects it by the process of abscission. Between the induction of senescence and abscission, the resources accumulated in the leaf are mobilized to young and growing parts. Various events associated with the mobilization process like degradation of macromolecules, interconversion of metabolites to their transportable forms, and their energy-dependent movement are tightly regulated. The remobilization of nutrients is a complex, extensive but highly co-ordinated process involving up-regulation of several genes (Figure 1). This is a mechanism that the plant has evolved to salvage the resources it has already acquired before the obsolete senescing leaves are cast off.

The expression of specific genes has been reported to be associated with the mobilization of breakdown products of the macromolecules during senescence. Different cDNA clones homologous to the genes for proteases as described earlier[6], like malate synthatase[13], glutamine synthatase[20] and ribonuclease[18] relating to macromolecular degradation and mobilization during senescence are isolated and characterized (Figure 1).

Mobilization of nitrogen

Several enzymes participate in protein breakdown and subsequent transfer of nitrogen. In addition to glutamate dehydrogenase, transaminase is known to be responsible for shunting of nitrogen into glutamine and asparagine, the common mobile forms of nitrogen[6]. The key enzyme responsible for the synthesis of glutamine is glutamine synthatase (GS1). For recovery of nitrogen from senescing leaves, GS1 plays a crucial role. The cDNA clones for GS1 have been isolated and their expression has been examined in detail during senescence of radish cotyledons[21]. It is suggested that the function of GS1 is

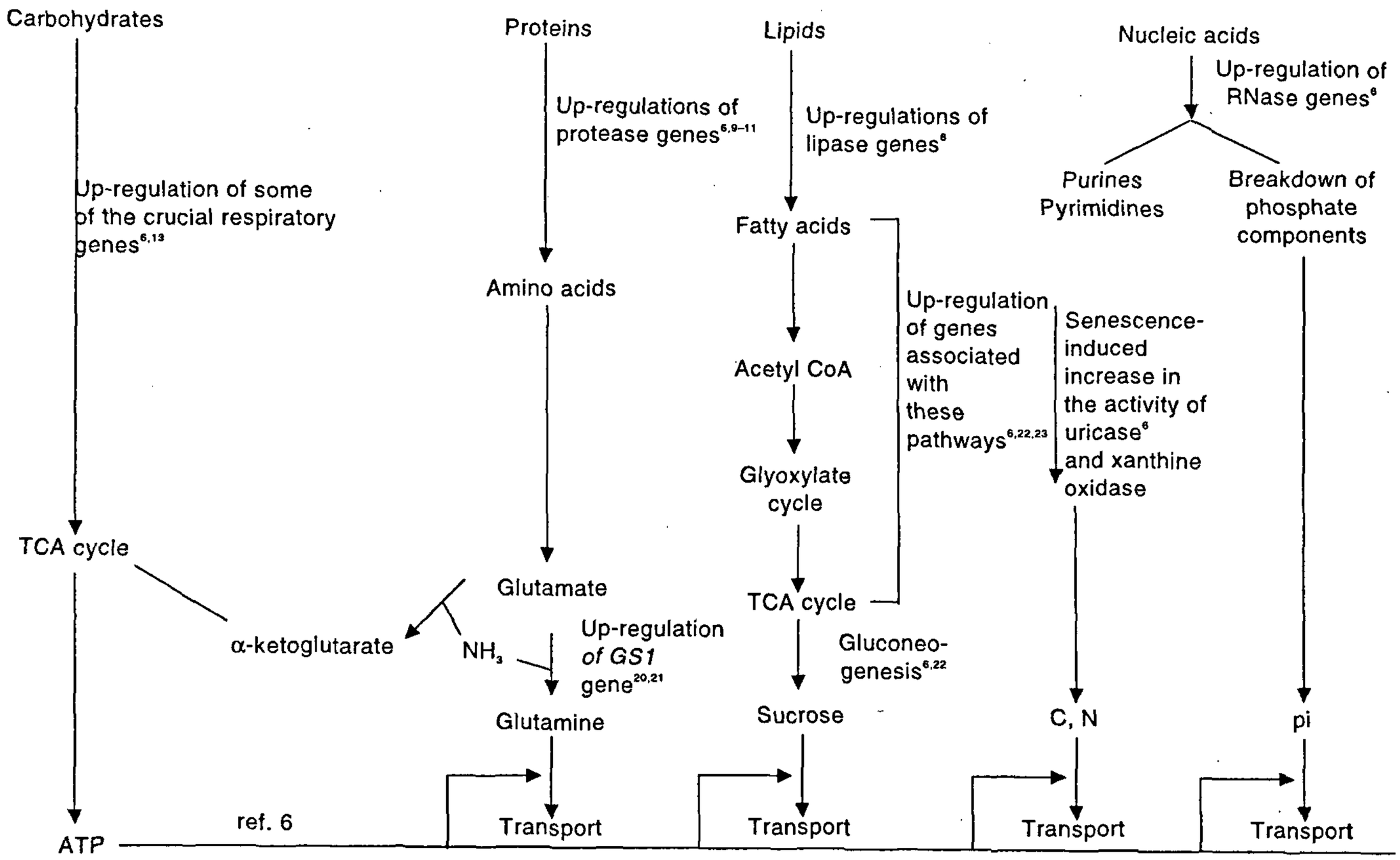

Figure 1. Up-regulation of several SAGs (senescence associated genes) associated with the degradation of macromolecules and transport of nutrients from senescing leaves. The transport involves metabolic energy, namely ATP. The energy level is controlled by up-regulation of genes associated with respiratory metabolism.

to convert ammonia to glutamine, a known transportable form of nitrogen that is remobilized from senescing leaves to growing parts of plants. Structurally three cDNA clones for the enzyme (GS1) have been characterized and two of them namely Gln 1:1 and Gln 1:3 have been shown to be expressed both during natural and dark-induced senescence as revealed by Northern blot analysis[21]. A cDNA clone homologous to GS1 has been shown to have enhanced expression during senescence in *Brassica*[10]. Its role has been attributed possibly in the synthesis of glutamine and subsequently its transport (Figure 1).

Breakdown of lipids, its conversion to sugars and their transport

The genes responsible for synthesis of the enzymes involved in gluconeogenesis are reported to be significantly expressed during leaf senescence[6]. A decline in photosynthesis during senescence may result in sugar starvation leading to the activation of conversion of lipids to sugars (Figure 1). Thylakoid breakdown leads to release of lipids, which are known to be converted to sugars through the glyoxylate cycle[6,22,23]. The sugars produced by conversion of large amounts of lipids may be in excess than that required for respiration of the senescing leaves and this may be exported to other growing and demanding parts of the plant in transportable form. It appears that the expression of genes for the enzymes participating in the process of gluconeogenesis for production of sucrose plays an important role during senescence[6,22] (Figure 1).

Mobilization of other nutrients

Leaf senescence also results in the breakdown of nucleic acids to purines and pyrimidines, which ultimately degrade to small and transportable carbon and nitrogenous compounds that are transported to growing parts of the plant[6] (Figure 1).

In addition to mobilization of carbon and nitrogen, other nutrients like sulphur and metallic ions are also known to be transported from senescing leaves. The polypeptide of a cDNA clone that has sequence similarity with plant ATP sulphurylase has been examined during senescence of *Brassica*[10]. The enzyme ATP sulphurylase is known to participate in the biosynthetic pathway of methionine and cysteine. During senescence,

its enhanced expression may modulate the level of cysteine and consequently its conversion to glutathione which plays a key role not only in minimizing the level of toxic oxygen free radicals produced during senescence but may also be involved in storage and transport of sulphur from senescing leaves to growing parts of the plants.

Genes involved in maintenance of cell integrity of senescing leaves

The progression of senescence involves energy and the process needs retention of transcriptional potential for the expression of senescence-related genes. The leaves also develop an efficient mechanism to minimize toxic levels of free radicals and maintain a level of cell viability. Since the process is basically deteriorative in nature, the senescing cells should have adaptational strategy to counter certain specific deteriorative events in order to maintain respiratory metabolism, protect the cells against the formation of free radicals and keep the trascription machinery active. Senescence-induced increase in the level of expression of a cDNA clone having sequence similarity with catalase gene, expression of the genes against pathogen attack and up-regulation of the genes against metal toxicity support this proposition[6]. The enhanced expression of genes for the synthesis of enzymes participating in the respiratory process during senescence is discussed elsewhere in this review. Lipid conversion to sugar is a major event favouring the proposition of energy maintenance of senescing leaves[6] (Figure 1).

Gene expression against infection

During leaf senescence of *Brassica*[10,24] and *Lycopersicon*[25], a significant increase in the expression of homologues of pathogen-related (PR) genes has been observed. The precise function of the products of PR genes during senescence still remains unclear. However, PR proteins are known to protect the plants against pathogen attack and genes for the proteins are therefore, known to be expressed during pathogen infection. Since senescing leaves are relatively prone to pathogen attack, the PR protein may protect the leaves against infection. On the other hand, some of the PR genes are also known to be regulated by plant developmental factors. In addition to leaf senescence[25], reports are also available on expression of the genes in germinating and developing seeds[26,27]. Supporting the proposition, Hanfrey *et al.*[24] have shown genes similar to previously known PR genes expressed during early stages of senescence of leaves not infected by any pathogen, suggesting a function of the genes not necessarily related to pathogen infection. The genes might be playing a role in a developmental signal transduction pathway associated with triggering of senescence in addition to their role against infection.

Gene expression against free radical-induced damage

Ferritins are iron-binding proteins that can store iron atoms and make them available in soluble and metabolically useful forms. The metabolic significance of senescence-induced increase in the expression of homologues of ferritin genes as reported by Buchanan-Wollaston and Ainsworth[10] in *Brassica* may suggest that during senescence, the degradation of some of the cellular macromolecules may result in release of free metals and consequently an increased pool of free iron. The free iron is known to bring about a metal catalysed reaction[6] for production of oxygen-free radicals, leading to the damage of senescing cells. The possible role of ferritin could be its participation in the formation of a complex with iron and transport of iron from senescing leaves to growing parts of the plant[6]. In a similar way, the significance of an enhanced expression of homologues of metallothionin genes as reported in *Arabidopsis*[28] and *Brassica*[10] could be explained. During senescence, metallothionin may bind with free metal ions released from protein breakdown and thus make them available for storage and transport. In the process, the production of free radicals catalysed by the free metal ions is minimized. In spite of these adaptational mechanisms, the senescing leaves also develop other kinds of strategy to minimize the level of free radicals including toxic oxygen radicals, which are significantly increased due to many factors including a loss in the activity of superoxide dismutase[29]. Senescence-induced enhanced expression of the catalase gene as reported by Thomas and de Villiers[28] and Buchanan-Wollaston and Ainsworth[10] may help in reducing the pool of these radicals.

Degradation of the chloroplast, its significance and its possible role in initiation of leaf senescence

Chloroplast dismantling

Chloroplast in a green leaf is the earliest and major target of senescence-induced catabolism[3,4]. Changes in the structural organization and function of chloroplasts have been extensively investigated during the process in many laboratories in various plant systems[1,2]. The organelle exhibits both quantitative and qualitative changes in the pigments[29,30], macromolecules[3,31], molecular structure[32,33], thylakoid organization[34] and in the enzymes that participate in carbon dioxide fixation in stroma[7,35,36]. The disassembly of thylakoid membranes

includes unstacking of grana followed by breakdown of membranes to plastoglobuli in addition to loss in primary photochemical reactions and breakdown of Rubisco, a major protein of green leaves[37].

Molecular mechanism

The proteases responsible for degradation of organelle-located proteins are yet to be characterized in detail, although several endopeptidases in chloroplasts have been reported by several authors[1,38,39]. It is possible that the initial steps for cleavage of these proteins occur in the organelle itself. Further, the presence of proteases in chloroplasts, degradation of proteins in the isolated organelle[40,41] and the report on degradation of SSU of Rubisco in the chloroplast by a proteolytic system encoded by a nuclear gene[42] may indicate action of the organelle protease in degradation of its protein. This is further supported by the observation of ClpP and ClpC protease subunits in chloroplasts of *Arabidopsis*[39]. The expression of chloroplast gene with sequence homology to the catalytic subunit of ATP-dependent bacterial protease ClpP has been observed. Secondly, leaf senescence is reported to induce expression of early responsive dehydration gene (*erd1*) encoding a protein with similarity to regulatory ATPase subunit (ClpA) of Clp protease[43]. ClpA interacts with ClpP synthesized in chloroplasts and brings about the degradation of proteins during leaf senescence[43]. However, the proteolytic degradation of chloroplasts during senescence has to be considered in the background of participation of vacuolar protease. Reports are available on the degradation of pigments and proteins of chloroplasts by vacuolar enzymes[44].

The question being presently addressed is what really initiates dismantling of chloroplast during senescence? Experimental evidences may suggest the degradation to be initiated by nuclear genes. A delay in senescence in nuclear gene mutants, retardation of the process by nuclear specific RNA and protein synthesis inhibitors and retardation of chlorophyll loss in isolated chloroplasts or anucleate cells[1,2] support this proposition. On the other hand, the non-nuclear gene responsible for senescence has been reported to reside in the chloroplast itself[45]. It appears that we may have to wait for the answer.

Significance of chloroplast degradation and induction of leaf senescence

Chloroplast catabolism plays a key role in the resource mobilization in plants. During senescence, it is the major source of carbon and nitrogen ultimately released during seed formation. About 90% of nitrogen exported from the organelle are Rubisco in stroma and light harvesting chlorophyll-binding proteins in thylakoid membrane[46]. The genes for these proteins and other photosynthesis-associated genes (PAGs) are known to be up-regulated during leaf development and expansion. However, during transition in the functional behaviour of the leaf when it switches over from a photosynthetic organelle to a storage organelle for transport, these genes are down-regulated[5,8,25,47–51]. The expression of two photosynthetic genes for Rubisco small subunit (rbcs) and Chl*a*/*b*-binding protein (cab) were extensively examined by Hensel *et al.*[8] during leaf senescence of *Arabidopsis* along with two marker genes associated with senescence that are up-regulated. These authors proposed a model suggesting the possibility of a down-regulation of photosynthetic genes as a cause for induction of leaf senescence and up-regulation of the genes for macromolecular degradation and their subsequent transport. A decline in photosynthesis intrinsically by a developmental signal or by various biotic and abiotic stress factors may initiate the senescence process as shown in Figure 2.

Although it is difficult to quantitatively find the threshold of photosynthetic decline that triggers foliar senescence, the compensation point when the leaf loses its potential to contribute the fixed carbon to other parts of the plant body, could initiate the process of senescence[52].

Molecular mechanism of foliar senescence

Leaf senescence as a programmed cell death

Recently, attempts were made to extrapolate the molecular mechanism of programmed cell death (PCD) of ani-

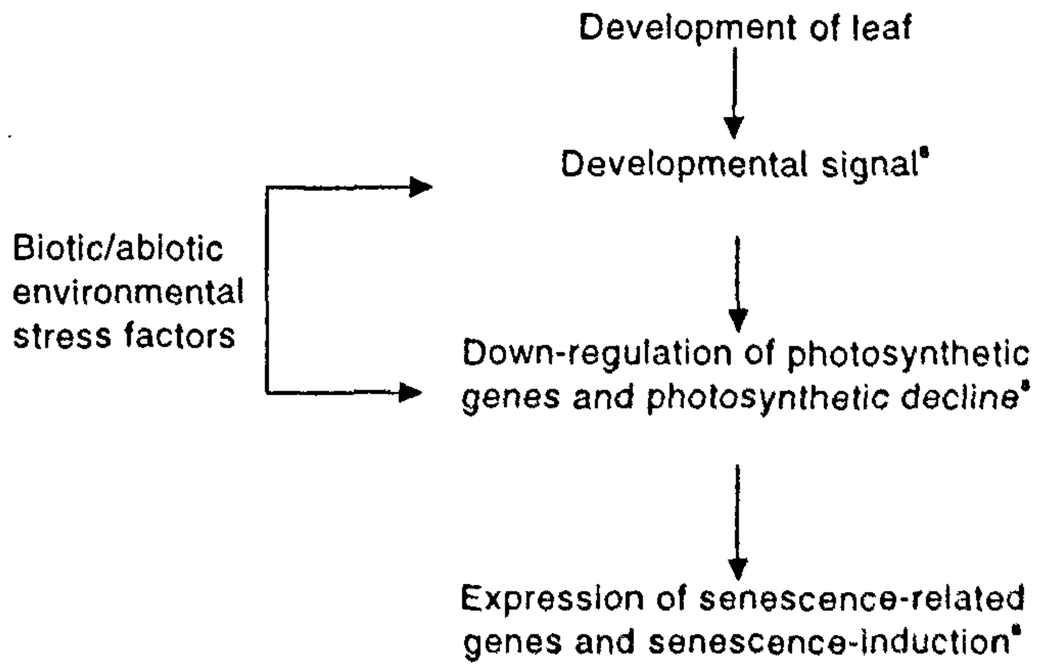

Figure 2. Initiation of leaf senescence by down-regulation of photosynthetic genes. A decline in photosynthesis by biotic/abiotic stress factors may directly or indirectly through modulating the developmental signal, cause induction of senescence.

mals in understanding the molecular events associated with senescence and cell death in plants[51,52]. Apoptosis in certain vertebrate tissues as characterized by diagnostic features of PCD like activation of endonuclease and cellular shrinkage is comparable with shrinkage of the cytoplasm and nucleus with fragmentation of DNA leading to cell death induced by low cell density in carrot cell suspension[53]. Similarly, the formation of tracheary elements of xylem has been used as a plant model of PCD[54]. When tracheary elements mature, they lose their nuclei, cell contents and form a hollow tube with secondary cell wall thickening. These events involve participation of genes suggesting occurrence of PCD[54]. The PCD during the xylogenesis with loss of nuclei, however, may be different from the programmed senescence of many other tissues where the nuclei largely remain unaltered during senescence.

The organized physiological, biochemical and genetic events exhibited by the developmentally-mediated senescence in leaves leading ultimately to death, imply that the process is programmed. The expression of specific genes and synthesis of cascade of proteins during induction and progress of the process may clearly recognize leaf senescence as a type of PCD, which appears to share many common points with PCD in animals[52]. On the other hand, apoptosis, a specific category of PCD might have a different logic in plants. During senescence, the disappearance of chloroplast DNA, the central characteristic of apoptosis has been observed in plants[52]. However, it is not clear whether the loss of DNA is really apoptic in nature or it has a physiological significance in the background of mobilization of phosphorus, a breakdown product of the nucleic acid from senescing leaves to other growing parts of the plants. However, a clear understanding of the PCD path during leaf senescence and its significance in plant life need further investigation.

Signals for expression of senescence-associated genes

The possible operation of signals from developing sink, hormone signals and changes in the level of metabolites regulating expression of senescence-associated genes have been suggested[6]. However, studies on signal perception, processing and expression of genes for initiation of senescence in leaf cells leading to death are meagre[6]. A reduction in the level of cytokinins, a possible hormonal signal may lead to induction/enhancement of foliar senescence which has been shown to be significantly retarded by its exogenous application or its overproduction in transgenic plants[51]. Similarly, the metabolism of ethylene during fruit ripening has been extensively examined[6,51]. The hormone is known as a signalling molecule for fruit ripening and its inhibition by antisense technology leads to retardation of ripening. The possibility of ethylene as a signalling molecule regulating foliar senescence has also been suggested[6,51], which may indicate a common signal transduction pathway of PCD both during senescence and fruit ripening. The other type of signalling system, extensively examined for induction of foliar senescence, is associated with the development of reproductive structures in plants. The onset of reproduction may produce a senescence-related signal that is transported to leaves for senescence induction[55]. Jasmonic acid has been suggested as a possible candidate, which of course needs confirmation with strong experimental support[6]. Recently, an attempt was made to characterize signalling mechanisms in a relatively simple system, namely single cell suspension of carrot cells, where PCD pathway is induced by low cell density[53]. The authors suggest that plants can regulate PCD as in the case of animals by a kind of social signalling system. The possibility of involvement of Ca^{2+} and protein phosphorylation in this signal transduction pathway has been discussed.

All these signalling systems, in addition to the down-regulation of photosynthesis as a possible signal for induction of senescence-related genes as discussed in previous section, are discussed in different plant systems in different experimental conditions. It is, therefore difficult to generalize the data in this area for emergence of an integrated picture of signalling system. It is, however, possible that these signalling systems with different pathways may meet at a common point leading to down-regulation of photosynthetic genes that trigger foliar senescence.

Regulation of gene action

The induction of leaf senescence is suggested to be associated with down- and/or up-regulation of several genes[8]. A few marker genes specifically expressed during senescence are also reported[6,8,12]. The question is, what does really control the switching on or off of these genes? Although a little is known about the precise changes that lead to the expression and/or suppression of SAGs accompanying senescence, recent experiments and analysis of promoters of some of the SAGs reveal *cis*-acting elements of the promoter sensitive to senescence-inducing agents. Chung *et al.*[56] have worked on the activity of the promoter of a senescence-associated gene (*sen1*) of *Arabidopsis*. The promoter of the gene was well characterized. It was fused with GUS, a reporter gene and then introduced to tobacco plants to investigate the activity of the promoter to senescence-inducing factors like darkness, variation in the sugar level and ABA treatments. The promoter activity was assessed by measuring the GUS gene product. Darkness, exogenous addition of ABA and sugar starvation in-

duced by DCMU treatment are shown to significantly promote GUS expression, suggesting that these senescence-inducing agents might be operating through signalling systems to regulate gene expression through this promoter.

On the other hand, the genetic regulation of retarding action of leaf senescence by cytokinins has been examined. Gan and Amasino[51] have used the promoter of a senescence-specific gene for expression of IPT, the enzyme catalysing the first controlling step of cytokinin biosynthesis. The novelty of their work is that the activity of the promoter depends on the onset of senescence in leaves. The promoter, therefore, activates production of cytokinins only when leaves start experiencing senescence. Production of the hormone prevents furthering of the process, which brings about attenuation of the promoter and thus prevents an excess accumulation of the hormone. Further work in this area may provide clues about the nature of *cis*-acting elements of senescence-associated genes and their interaction with transacting factors and thus reveal the story of signal response coupling during senescence.

Conclusions and perspectives

The study of leaf senescence at the molecular level is rather recent. In spite of the significant progress made in plant molecular biology, several questions remain unanswered in understanding the process, some of which are addressed here:

(i) Many cDNA clones relating to leaf senescence are isolated and the clones are identified on the basis of sequence homology with the proteins and nucleic acids in the database search. Although their functions are extrapolated with known functions of the genes, the implications of the function in understanding the mechanism of induction and progress of senescence still remains unclear. Secondly, we have failed to identify some of the cDNA clones very specific to senescence process because of lack of sequence homology of these clones. With advancement in plant molecular genetics, particularly in the area of sense and antisense transformation of plants, we should be able to have further insight into the nature of gene products and their metabolism that may help in understanding the mechanism of senescence.

(ii) Although the genes associated with leaf senescence are isolated and characterized, the precise nature of the regulation of their expression is not clearly understood. The analysis of promoters of many senescence-related genes reveal no uniformity in sequence elements, which would suggest that regulation of these genes during the process is complex and is controlled by several factors through different signal transduction pathways. Some progress has been made in isolation and characterization of the promoters of a few SAGs. But identification of the transacting factors and their interaction with promoter sequences for regulation of the process are not successfully investigated yet.

(iii) Not much is known about the nature of the signals and their transduction in induction of leaf senescence. Because of the complex nature of genetic regulation of the process, it is not yet possible to identify the signal transduction pathways between the perception of specific signals and initiation of cascades of gene expression and finally the phenotypic expression of the senescence syndrome. Secondly, information on the nature of initial signals and their transduction, whether the same or different in induced and natural senescence are not available. The nature of signals in the senescence process induced by the environmental stress may be different from that of the natural age-dependent process. In monocarpic plants, the initiation of leaf senescence and development of reproductive organs appear to involve a tight correlation possibly controlled by a co-ordinated signalling system. However, the precise nature of biochemical routes of signal processing is not unequivocally established.

(iv) Chloroplast is one of the major targets of senescence-induced degradation. In fact, a major amount of nitrogen to be exported from senescing leaves comes from the organelle. However, the mechanism of protein degradation in chloroplast and identification of proteases responsible for degradation of the macromolecules are yet to be known.

(v) A good plant model for the study of leaf senescence is still lacking. The cells in a single leaf never get senescent uniformly and simultaneously. Although dark-induced senescence and senescence of excised leaves provide a good model so far as cell synchrony is concerned, these models have their own limitations. The problem of choosing a better system for the study of molecular biology of leaf senescence still remains unsolved.

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